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Regulation of ferulic catabolic genes in *Pseudomonas fluorescens* BF13: involvement of a MarR family regulator

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Abstract In *Pseudomonas fluorescens* BF13, the cluster of genes essential for degradation of ferulic to vanillic acid (*ech*, *vdh* and *fcs*) is expressed in ferulic but not in succinic-grown cells. In the upstream region, we identified a gene, *ferR*, encoding a protein homologous to transcriptional regulators of the MarR family. A *ferR* knockout mutant (BF13–89) showed a 3.5-fold increase in expression of an *ech*-reporter gene fusion compared with the parent strain in succinic-grown cells, indicating that the *ferR* gene product negatively regulates expression of the ferulic catabolic operon in *P. fluorescens* BF13. Consistent with the increased expression of the catabolic genes in the *ferR* mutant, BF13-89 showed a shorter (relative to its $FerR^+$ parent) lag phase during carbon source shift from succinic to ferulic acid. However, expression of *ech-lacZ* fusion did not increase in BF13–89 grown in the presence of ferulic acid, indicating that FerR has a second function as transcriptional activator. Expression of *ech-lacZ* in a feruloyl-CoA synthetase-deficient strain revealed unambiguously that FerR-mediated activation of the ferulic catabolic operon is dependent on the thioester product of the feruloyl-CoA synthetase reaction.

Keywords Ferulic acid catabolism · *Pseudomonas* · MarR family regulator · FerR · CoA thioester · Gene transcription

Introduction

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a ubiquitous plant constituent that is produced from the metabolism of phenylalanine and tyrosine. It is the main phenolic component found in cell walls of monocotyledons constituting about 1.4 g/kg in barley grains (Nordkvisk et al. 1984), 9 g/kg in rice endosperm cell wall (Shibuya 1984), 6.6 g/kg in wheat (Smith and Hartley 1983), and 31.0 g/Kg in maize bran (Saulnier et al. 1995).

There is a growing interest in the potential use of ferulic acid as feedstock for biocatalytic conversion into valuable compounds (Rosazza et al. 1995). Specifically, there is significant interest in microbial pathways of ferulic acid dissimilation that generate aldehyde intermediates, such as vanillin, the main flavour component of vanilla (Walton et al. 2000; Priefert et al. 2001; Schrader et al. 2004).

Six major pathways have been proposed for the initial degradation of ferulic acid, namely non-oxidative decarboxylation, side-chain reduction, coenzyme-A-independent and coenzyme-A-dependent deacetylation, demethylation and oxidative coupling (as reviewed in Mathew and Abraham 2006). The genes and enzymes involved in the coenzyme-A-dependent, non-β-oxidative pathway, which is the most common pathway of ferulic acid degradation in bacteria (Priefert et al. 2001), have been characterized in *Pseudomonas fluorescens* AN103 (Gasson et al. 1998), *Pseudomonas* sp. HR199 (Overhage et al. 1999), *Pseudomonas putida* KT2440 (Plaggenborg et al. 2003), *Streptomyces setonii* (Muheim and Lerch, 1999), *Amycolatopsis*

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